RECEIVED
JAN 29
VET. MED. LIBRARY

OHIO STATE UNIVERSITY
JAN 22 1958
LIBRARY

THE JOURNAL OF THE BRITISH VETERINARY ASSOCIATION

The Veterinary Record

PATRON: HER MAJESTY THE QUEEN

VOL. 69 NO. 52 ISSUE NO. 4168 1445 - 1456 DECEMBER 28th 1957

End Barbiturate Anaesthesia–

within seconds

'Megimide' is the new barbiturate antagonist which dramatically reverses the hypnotic and respiratory depression produced by barbiturates.
Used after barbiturate anæsthesia, it immediately accelerates the return of reflex activity and normal respiration, and is invaluable in effectively lightening barbiturate anæsthesia and shortening the post-operative period of recovery.

Clinical Comments

"In these experiments (with 20 cats and 60 dogs) we have shown that Megimide can be used in veterinary anæsthesia for healthy dogs and cats. Full surgical anæsthesia is immediately terminated, and the animals usually awaken."
(*Vet. Rec.*, 1957, 69, 304.)

"Our experiments showed that Megimide successfully roused these animals (cats) within a matter of minutes. . . . Megimide is a most valuable adjunctive treatment of anæsthetic emergencies, and its use is recommended."
(*Can. Jnl. Comp. Med. and Vet. Science*, 1957, 21, (5), 165.)

'MEGIMIDE' TRADE MARK

The New Barbiturate Antagonist

Literature supplied on request

A. & G. NICHOLAS LTD.

Ethical Pharmaceuticals,
SLOUGH, BUCKS, ENGLAND

The
VETERINARY RECORD

Founded by William Hunting, F.R.C.V.S., in 1888

7, MANSFIELD STREET, PORTLAND PLACE, LONDON, W.1. MUSEUM 6541

No. 52 DECEMBER 28th, 1957 Vol. 69

Registered for Transmission as a Newspaper *Price 2s. post free*

Symposium on Viruses*

BY

J. B. Brooksby; T. W. F. Pay; W. F. H. Jarrett; J. T. Stamp; H. P. Chu; J. G. Campbell

Some Aspects of the Serology of Viruses

BY

J. B. BROOKSBY
Research Institute (Animal Virus Diseases), Pirbright, Surrey

THE serology of viruses is of interest to the research worker mainly for the light it can throw on problems of virus structure and virus-host relationship. Serological techniques used in diagnosis of disease have their own interest but an account of methods for this purpose can be found in the appropriate text-books. This paper is therefore confined to 3 main aspects which are of primary importance in the serology of virus from the more fundamental point of view. These 3 main aspects are:—

1. The peculiarities of viruses as serological antigens.
2. The information that serology gives on structure and multiplication of viruses.
3. The significance of specificity of serological reactions among viruses.

(1) The Peculiarities of Viruses as Serological Antigens

Viruses have disadvantages as serological antigens as compared with both protein molecules and bacteria. Protein solutions give satisfactory precipitation reactions because of the high molecular concentration of small particles. Bacterial suspensions show agglutination because of the clumping together of lower concentrations of bigger particles. Viruses have neither the high concentration of the protein nor the large particle of the bacteria.

One of the earliest considerations of the problem of size and concentration of antigens in relation to their serological activity was made by Merrill (1936). He plotted the relation between size of particle and concentration (particles per ml.) necessary for visible precipitation. He was not able to obtain experimental points for any viruses smaller than vaccinia but his deductions for viruses such as influenza and poliomyelitis are not widely different from those which have later been obtained by experiment using newer techniques for concentration of the viruses. Success with flocculation has been reported even with some of the smallest viruses. Recently, Wilson Smith and his co-workers (1956) have demonstrated flocculation with ultracentrifugal concentrates from tissue culture preparations of the virus of poliomyelitis. The infective titre of these is lower than might be expected.

Another approach to the demonstration of precipitation is the agar gel diffusion method which has given very good results with a number of animal viruses, notably those of the pox group, influenza and, in the veterinary field, with myxomatosis and swine fever and with foot-and-mouth disease. Perhaps the most elegant demonstration of type specificity in foot-and-mouth disease is given by the work of Brown and Crick (1957). Cups of 2 mm. diameter, 8 mm. apart are cut in a gel prepared with washed agar; serum is used in a dilution of 1 in 4 in the outer ring of cups and virus consisting of undiluted vesicle fluid or a concentrate of epithelial suspension placed in the centre cup. After 24 to 48 hours at 25° C., lines of specific precipitation are visible. The success of this method is probably due to the fact that the diffusion gradient of virus and antiserum in opposite directions ensures that there will be a point at which the two reagents will be present in optimal proportions. The application of the method in the

* Six papers presented to the Association of Veterinary Teachers and Research Workers on March 2nd, 1957. Mr. J. T. Done's paper on "Rhinitis in Pigs" is being published in a more specialised journal.

virus field may be of great use both for fundamental studies and for antibody titration. Already in the field of protein chemistry antigenic relationships have been extensively studied.

I have dealt so far with the direct observation of precipitation and flocculation. The emphasis here has been on the necessity for large amounts of virus. For more ready detection of an antigen, it is obvious that some device to multiply the scale of a reaction must be employed and this in fact is the aim of most of the remaining methods in *in vitro* serology. It may be useful here to indicate the relative sensitivity of the various tests:—

Complement fixation consists essentially in removing a protein from the test solution and determining the quantitative removal by back titration with a gross reaction involving erythrocytes. It has a sensitivity of 10 to 100 times that of direct flocculation.

Haemagglutination, which depends on the reaction of virus and cell receptors, is highly sensitive, perhaps more so than complement fixation. It is not a true serological method since no antiserum is involved and, of course, is limited in scope by the characteristic property of particular viruses to become linked to red cell receptors. Only a limited number of viruses have this property. Agglutination of coated red cells, bacteria or collodion particles has been used. The sensitivity of this reaction is not generally greater than that of complement fixation and the difficulty of preparation of coated particles is often the main obstacle.

Another phenomenon which has been applied to viral serology is the *Immune Adherence Phenomenon* of Nelson. This depends on the tendency of primate red cells to agglutinate in the presence of mixtures of antigen and antibody. It has been applied so far to a limited number of viruses of the middle and larger size. With the smaller viruses such as poliomyelitis and foot-and-mouth disease, it has not been possible to obtain regular results. Virus concentrations of about 10^8 particles per ml. are necessary so that the method is not so sensitive as complement fixation.

The conclusion from the evidence is, therefore, that Merrill's figures indicate roughly the likelihood of success with techniques at present available. The mass of virus material must be substantial before serological methods are likely to be successful. This does not always mean that high infectious titres must be obtained. Serological activity can be associated with non-infective particles, and even with matrix substances derived from infective tissue, scaffolding as it were of the process of virus replication. It would seem that serological methods are worth trying even in the face of the odds which theoretical conclusions suggest.

(2) Serological Information on the Structure and multiplication of virus

The first evidence on virus structure, other than that of size of infective particles determined by ultracentrifugation and ultrafiltration, was the detection of so-called soluble antigens. At the time this evidence was not in fact considered as evidence on structure, for the soluble antigens were thought of, by analogy with the bacteria, as something in the nature of flagellar antigens as opposed to somatic antigens of the whole particle. The detection of soluble antigens however, was gradually extended from work with the larger viruses such as vaccinia to smaller viruses such as mumps, influenza and Newcastle disease, and finally to the very small viruses such as poliomyelitis and foot-and-mouth disease. Now as the size of the virus decreased, the size of the soluble antigen did not decrease proportionately until with the smaller viruses, for example foot-and-mouth disease, where the most precise measurements have been made, the large particle and the small differ by only a factor of 3 in diameter. It was then abundantly clear that the small component was an integral part of the large, and the possibility of structural hypotheses began to exercise the theoretical virologist.

The Virus of Foot-and-Mouth Disease

In foot-and-mouth disease the first study in which serological activity as well as infectivity was studied, was made in 1943 by the German workers Traub and Pyl, and Mohlmann. The subject was reinvestigated by Bachrach in 1952 but in common with the earlier workers he did not go beyond the statement that on ultracentrifugation, infectivity could be reduced by 99 per cent. or more while the reduction on complement fixing activity would be only slight, say by 2- or 3-fold. The conclusion was that complement fixing activity was associated with a soluble antigen, but none of these workers made any attempt to estimate the size of the smaller particles involved. Bradish, Brooksby, Dillon and Norambuena (1952) dealt with more accurate studies in the ultracentrifuge coupled with a more precise estimation of both infectivity and complement fixing activity. It was found that complement fixing activity was associated with two particles, one removed at the same time as infectivity and the other sedimented much more slowly. More accurate determination of the sedimentation properties in radial tubes allowed estimation of the size of the components involved as 20 mμ and 7 mμ respectively. For typical fresh preparations of virus from cattle and guinea-pigs, the smaller particle accounted for about 50 per cent. of the complement fixing activity of the original preparation and for certainly not more than 1/10,000 of the infectivity. The larger particle is the major infective component and has associated with it the remaining 50 per cent. of the complement fixing activity.*

The next part of the work was prompted by the finding that in samples of virus stored at −20° C. for 2 to 3 months the proportion of complement fixing activity associated with the small particle reached 90 to 100 per cent. Storage under these conditions of course results in some loss of infectivity and this suggested that the large component

* Dr. Bradish and I have now considerably extended this work. The remaining studies described in this section on foot-and-mouth disease have been made with his collaboration.

had broken down and disappeared from the system. It therefore became of interest to observe the effect of treatment known to inactivate virus or to destroy infectivity. Heating at temperatures up to 56° C. was used for this purpose but other methods may be equally suitable. Typical experiments on heat disintegration were carried out as follows; first the two components were separated by two cycles of ultra-centrifugation. The complement fixing activity of the original was distributed equally between the two fractions. On heating the activity of the supernatant fell by 40 per cent. while the deposit increased by 40 per cent. The result indicates liberation of new complement fixing activity on the heating of deposit fractions. The mechanism involved has not as yet been fully investigated. The first explanation was simply the breakdown of large particles to yield small ones; but this does not give the complete picture. Attempts to determine the size of the complement fixing activity of the deposit material gave sedimentation properties closer to the small component than the large, yet infectivity determinations suggest that the large component is still present in unheated deposit material and this is confirmed by electron microscopy. Before heating, particles of size 20 mμ or so are seen, while after heating the characteristic particle disappears leaving small granular material of about 7 to 8 mμ diameter. Two possible explanations of the appearance of small centrifugal activity in unheated deposit material can be given. First, it may be that dissociation takes place at the surface of the large particles, liberating the small centrifugal component but leaving the large particle more or less undamaged. The second hypothesis is that a proportion of large particles undergo disintegration and sufficient small complement fixing activity is liberated to give a sedimentation co-efficient about that of the small component. The characteristics of the materials remaining after heating are quite clear. Infectivity has been destroyed and large particles have been disintegrated. The complement-fixing activity is associated with material of similar sedimentation co-efficient to that in unheated supernatant material.

Although specificity is discussed more particularly in the next section, I should like here to refer to our studies on specificity of the fractions. Although differentiation of the immunological types of foot-and-mouth disease is probably as distinct as that between the types of any single virus known, there is still some cross reactivity between the types in cross-complement fixation tests or cross-serum neutralisation tests. Such cross reactions are more obvious with some strains than with others and may be so great in a few cases that in complement fixation tests it is difficult to assign a strain to the correct type group on these tests alone. Working with such strains it was found that the specificity of the large component fraction was greater than that of the small. The large component fraction is completely type specific, while the small is responsible for almost all the heterologous fixation of the original material. This phenomenon can also be observed with the stock type guinea-pig strains using high titre sera. A further observation has been made by applying heat treatment to the fractions obtained. Disintegration of the large component results in loss of specificity, as the large material is broken down to small.

Summarising, the picture that has emerged from these studies of the virus of foot-and-mouth disease is as follows:—

From infected tissue suspensions can be sedimented large particles of 20 mμ (approximately) in diameter which are infective. These particles have a highly specific serological activity, even though a small serologically active component seems to appear almost at once in the fraction. On heat treatment the large particles disintegrate giving rise to increased serological activity and at the same time the sharp specificity disappears. The heated product resembles, in size of particle and specificity, the natural small component. The natural small component is present in infected tissue suspension. It has a particle size of 7 to 8 mμ and a low degree of specificity.

The Virus of Influenza

It is not possible in the time available to deal fully with the cases of the other viruses for which information of this type is available. Most work has been done on influenza where soluble antigen S and viral antigen V have been studied—in this country notably by Hoyle, in the United States by the Henles and in Australia by Burnett. The two antigens are differentiated more clearly than those in foot-and-mouth disease; the small antigen is type specific only, while the larger one is, in addition, strain or subtype specific. Specific sera against the two antigens are prepared more readily than with foot-and-mouth disease and the occurrence of antibody to each antigen varies with the kind of sera studied, thus sera from vaccinated individuals react with V and convalescent sera with V and S; this is not so with foot-and-mouth disease. There has been much discussion on the rôle of S antigen in multiplication of the virus. Hoyle believes it to be the self-replicating unit of the virus but other workers are more cautious and suggest that it may even be a matrix substrain and attached only adventitiously to the virus particles. There is an interesting parallel with the findings in foot-and-mouth disease in that some workers differentiate between the small antigen obtained naturally and that from particles disintegrated by ether treatment. Ether treatment has been used to free the components from influenza. Hoyle suggests the structure of an influenza particle as a mass of soluble antigen particles in a lipoid envelope. Disintegration sets free both soluble antigens and spherical haemagglutinating particles of size 30 mμ, intermediate between the infective particle (70 mμ) and soluble antigen (10 to 15 mμ).

The Virus of Poliomyelitis

Current work on poliomyelitis clearly indicates a small serological antigen in the virus system. The particle size of the whole virus is in the region of 24 to 30 mμ, while the small antigen is in the range

of 8 to 12 mμ. Again, as in influenza, there is a difference in the occurrence of antibody to the different antigens in sera of different origin. South African workers claim that virus passaged in adult mice produces sera against only large particles, while that passaged in suckling mice produces sera against both small and large. The same workers claim to have demonstrated infectivity associated with particles of two different sizes, 24 mμ and 30 mμ, by collection of samples at different levels in the tubes of the ultracentrifuge. A parallel between poliomyelitis and foot-and-mouth disease is that the strain sensitivity of heated virus on complement fixation is less than the unheated virus, suggesting disintegration of large particles in a similar manner.

Other Viruses

Work on separable antigens has also been done with the viruses of Newcastle disease, rabies, blue tongue, Rift Valley fever and African horse sickness and vesicular stomatitis. In all cases complexity has been demonstrated, and frequently the small complement fixing antigen has been found to be of 8 to 12 mμ in diameter. In blue tongue, strain differences can be demonstrated by using saline antigens but not with ether treated (disintegrated) antigens.

(3) Serological Specificity among the Viruses

The serological specificity of viruses can be dealt with under 3 main headings: disease, type and subtype.

The first, *disease specificity*, is not of great importance as a serological problem. The distinction between different viruses is usually very sharp and differentiation of viruses on serological grounds presents little difficulty. There are a few cases where serological methods have been used in differential diagnosis of clinically indistinguishable diseases. Perhaps the best instance is of three conditions affecting swine—foot-and-mouth disease, vesicular stomatitis and vesicular exanthema—which are readily differentiated by complement fixation.

The second specificity, that of *type*, is assuming greater importance as the list of viruses showing major type differences lengthens. The earliest work on type differences depended on cross-immunity experiments but latterly serological methods have been responsible for extensive classifications without recourse to cross-immunity. Good examples are provided by the Coxackie group of viruses, the adeno viruses, blue tongue and African horse sickness. The type difference is of course less distinct than the disease difference, but it is still sufficient for simple serological methods to give satisfactory results. It must be realised that there is frequently evidence of a group antigen, common to the various types of an individual virus, and unless the technique of the test is carefully controlled this group antigen may interfere with type specificity. Thus, in foot-and-mouth disease using an appropriate complement fixation test, there is reasonably good differentiation of type; but by altering the conditions of the test, almost as good fixation can be obtained with heterotypic serum-virus mixtures as with homotypic. This result is obtained by mixing virus and serum before complement is added. It is probable that viral disintegration is the factor responsible for this result. These observations emphasise the need for care in interpreting the results of serological tests. The use of a variety of different methods avoids misinterpretation of results, for in this case of a heterotypic virus-serum mixture which fixes complement, there is no evidence of neutralisation of the virus involved to more than a slight degree. Similar mixtures of virus and serum brought together in the agar gel diffusion test do not show lines of precipitation. The conclusion must be that the antigen-antibody complex formed in the heterotypic virus-serum mixture though capable of fixing complement, is of quite a different character to that formed in the homotypic mixture which of course is neutralised, and is capable of forming visible precipitates. The results quoted of course represent the extreme case and do not affect the usefulness of the routine complement fixation test in type differentiation of foot-and-mouth disease.

The third degree of specificity, that of *strain or subtype*, is of a lower order than that of disease or type specificity. The emphasis therefore, must be on quantitative accuracy in the tests used. Differentiation depends on detailed cross tests between strains and their homologous antisera, on a chess board basis, both by complement fixation and serum neutralisation. The importance of correlating the results of different serological tests and then linking the results to problems of immunity cannot be too strongly emphasised. Only correlation of the results on a quantitative basis will establish the epidemiological importance of particular serological findings. Such correlation is laborious work but more rewarding than the mere taxonomy of strains. As in the consideration of type specificity, so in the case of the subtypes, it is necessary to be cautious in interpreting the results of the individual methods. It is frequently said for example, that comparison of neutralisation tests and *in vitro* tests of specificity, is valueless. In some cases the methods used may not have been those best calculated to detect differences; thus heated antigens in poliomyelitis are not so strain specific as those which have not been heated. In the adeno viruses, complement fixation tests with heated antigens do not allow of strain differentiation while serum neutralisation tests with the same sera are sharply specific. On the other hand good correlation between neutralisation and other serological tests has been obtained in foot-and-mouth disease and influenza. The *in vitro* test can therefore, be of great help in the classification of subtypes. The work at Pirbright on Mexican strains of type A foot-and-mouth disease, suggested that not only was the qualitative difference between strains indicated by cross-neutralisation, cross-complement fixation and cross-vaccination tests, but also that the quantitative differences were of a similar order in the tests used. There is not time to make more than passing mention of the influence of the antiserum in these reactions, but it is essential to remember that specificity, as indeed all other immunological properties, depends equally on the antigen and the

antibody. There is considerably evidence of the formation of antibodies to fractions of the virus (the V and S antibodies in influenza have been referred to earlier). There is also evidence of group antibodies related to a number of types of a particular virus. Sometimes these group and type antibodies can be differentiated by electrophoresis.

The type specificity of sera may vary with the progress of convalescence. The initial response to infection in foot-and-mouth disease is productions of antibody which may neutralise all types of virus almost equally. Such antibody is found in sera up to 10 days or so after infection and it is only in the next 10 days that heterotypic neutralising power declines while homotypic goes on increasing in titre. It may not be possible by neutralisation tests to determine the infecting type of virus by examination of serum samples taken in the period up to 10 days convalescence (Skinner, 1953).

This paper has covered a wide field with little detail. Serology is one of the techniques which has not yet been fully exploited in the study of viruses. It is thought of too often, as Heidelberger has put it, as a Diagnostic Drudge, but in the last few years it has contributed much to the better understanding of the virus system.

References

BACHRACH, H. L. (1952). *Amer. J. Vet. Res.* **13.** 13.
BRADISH, C. J., BROOKSBY, J. B., DILLON, J. F., & NORAMBUENA, M. (1952). *Proc. Roy. Soc., Ser.B.* **140.** 107.
BROWN, F., & CRICK, J. (1957). *Nature.* **179.** 316.
MERRILL, M. H. (1936). *J. Immunol.* **30.** 169.
MOHLMANN, H. (1943). *Z. ImmunForsch.* **103.** 265.
SKINNER, H. H. (1953). *Proc. XV Int. Vet. Congr., Stockholm.* **I.** 195, and **II.** 208.
TRAUB, E., & PYL, G. (1943). *Z. ImmunForsch.* **104.** 158.
WILSON SMITH, LEE, L. H., SHEFFIELD, F. W., & CHURCHER, G. (1956). *Lancet.* **i.** 710.

The Uses of Tissue Culture Methods in Foot-and-Mouth Disease Research

BY

T. W. F. Pay
Research Institute, Pirbright

FOOT-and-mouth disease besides being the first animal virus identified also can take pride of place in being among the first viruses to have been propagated in tissue culture. Hecke (1930, 1931) using plasma clot cultures and Maitland and Maitland (1931) using suspended fragment cultures of guinea-pig embryo tissue independently observed that foot-and-mouth disease virus could be propagated *in vitro*. Bibliographies of research on foot-and-mouth disease using tissue culture methods have been given by Murray and Kopech (1953) and Ubertini, *et al.* (1956).

Much of the considerable progress that has been made in recent years in the study of many animal viruses is directly attributable to the increased use of tissue culture methods. They have not only rendered possible a great amount of work with viruses, such as poliomyelitis, for which there is no inexpensive laboratory host, but they have also facilitated fundamental studies on cell-virus relationships, which previously were either more difficult or impossible to carry out in the infinitely more complex system of the experimental animal. The introduction of the use of unweaned mice as experimental animals for the titration of foot-and-mouth disease virus by Skinner (1951) has already done much to fill the gap which possibly now would have been filled in part by the use of tissue culture methods of titration. However, there are still many fields in the study of foot-and-mouth disease in which tissue culture methods are proving very valuable research tools. As most of the methods mentioned in this have been fully described elsewhere, it is intended here to outline some of the special uses to which they may be put rather than to describe the actual techniques in any detail.

Propagation of Virus

The method which has proved to be of the greatest economic importance to date is the large-scale culture of virus for vaccine production introduced by Frenkel (1947). This method is already in use in a number of centres in various parts of the world, and a plant for producing vaccine in this way has recently been commissioned at Pirbright. The details of the method have been fully described in a number of publications (Frenkel, 1947, 1953, 1954) (Mackowiak *et al.*, 1955) (Willems & Leunen, 1956). In brief, it consists of suspending slices of tongue epithelium, collected from cattle immediately after slaughter, in a suitable medium, adding a seed virus suspension and incubating the mixture at 37° C. for 18 to 24 hours. At the end of this time the tissue may have acquired a virus content comparable to that of material collected from cattle reacting to inoculation with the virus. Henderson and Galloway (1953) have shown that vaccines prepared from culture virus were as effective as those prepared from virus passaged in cattle. As these authors have indicated, the chief disadvantages for using cattle as a source of virus for vaccine production are their high cost, the necessity for having accommodation for large numbers of cattle under conditions that will prevent the spread of infection, and the difficulty of obtaining susceptible animals in countries where the disease is endemic or vaccination is extensively practised. With reference to the latter, it should perhaps be noted that the culture method can be used with complete success employing tissue from the tongues of vaccinated cattle.

More recently, Sellers (1955) and Bachrach *et al.* (1955) have shown that foot-and-mouth disease virus will multiply in cultures of pig and ox kidney. Wesslen and Dinter (1956) and Petermann *et al.* (1956) have confirmed these results and have suggested that the method is capable of development for the production of vaccine along lines similar to those currently used for the production of poliomyelitis vaccine.

Assay of Virus

The first method for the titration of foot-and-mouth disease virus based on tissue culture was that described by Brooksby and Wardle (1954). Suspensions of surviving bovine tongue epithelial fragments contained in cups in perspex plates were inoculated with dilutions of virus and incubated for a suitable

period at 37° C. The presence of viral multiplication was detected by a complement fixation test carried out *in situ* on the cultures in each cup. It was shown that with most of the strains examined the results agreed very closely with those obtained in cattle by tongue inoculation. These authors also showed that this method was usable for the detection of antibody by the serum neutralisation test. Gillespie and Frenkel (1955) described the successful use of tongue epithelium from vaccinated or convalescent cattle with this culture method. Van Bekkum (1955) showed that the results obtained by this method for the measurement of the antibody response to vaccination could be correlated with those obtained by the direct challenge of cattle with infective virus. Nardelli and Perini (1956) compared the sensitivity of this method for titration with that of mouse inoculation using virus strains of different origins.

Following the technique first described by Dulbecco (1952, 1954) and now widely known under his name, Sellers showed that foot-and-mouth disease virus would multiply and produce degenerative plaques in monolayer cultures of pig kidney (Sellers, 1955) and ox kidney (Sellers, 1957). By counting the number of plaques produced by a known dilution of a virus preparation, it is possible to calculate the infectivity of the original material. Results obtained by this method of titration have with many strains of virus agreed closely with those obtained by titration in mice, cattle or bovine tongue epithelium cultures. It has been shown recently (Sellers, 1957) that the sensitivity of this method is dependent upon a number of factors, which include both the composition of the culture medium and of the gas mixture above it. This author has also described a difference in the size of the plaques produced by certain strains of virus and it is hoped that this observation will prove of value as a marker in carrying out genetic studies on such virus strains. Furthermore, this method can be of great value for genetic and other fundamental virus studies in the ease with which pure lines of virus can be produced by the repeated isolation of virus from single plaques.

Besides using pig or ox-kidney monolayer cultures for the plaque counting method, it is possible to use them for titration by means of the limiting dilution of infectivity method (Bachrach *et al.*, 1955) (Wesslén and Dinter, 1956). Bovine tongue epithelium monolayer cultures containing the susceptible "membrane" type of cell growth have also been used successfully in this way (Pay, 1957).

Virus Multiplication Studies

Surviving cell cultures

Henderson (1953a, 1953b) using a modification of Frenkel's method carried out "growth curve" experiments with foot-and-mouth disease virus. He infected finely minced cattle tongue epithelium with high titre seed virus preparations, removed the bulk of unutilised seed virus by repeated washings, suspended the tissue, in Tyrode solution plus 10 per cent. v/v normal bovine serum and incubated it at 37° C. Samples of tissue and culture liquid were removed at intervals for titration of infectivity. It was found that a latent period of 2 to 3 hours was followed by a rise in infectivity of the tissue which reached a peak between 12 and 24 hours. The first increase in infectivity of the culture liquid could be detected only at about 5 hours when it had risen above the level of infectivity produced by the residual seed virus. Other workers (Mackowiak *et al.*, 1955; Ubertini *et al.*, 1956; Frenkel & Ribelin, 1956) using similar techniques have published results which do not differ greatly from the above.

This method, while yielding information of much value relevant to the large-scale culture of virus is of limited value for a more fundamental quantitative study of virus multiplication. Its main limitations are that the total number of cells which become infected is unknown and it is impossible to infect all susceptible cells simultaneously. These disadvantages were largely overcome by the use of suspensions of dispersed cells prepared by the action of trypsin on cattle tongue epithelium (Cartwright, Pay and Henderson, 1957). With this system total and differential counts of living and dead cells of the various types present were made; all susceptible cells were infected simultaneously with a high multiplicity of virus, extracellular seed virus was completely removed by repeated washings plus the action of specific immune serum, and the percentage of cells which subsequently became infected estimated by a titration method. Very briefly, the main features of growth curve experiments done by this method were that after a latent period of 2 to 2½ hours, the infectivity of the tissue increased, followed almost immediately by release of virus into the culture liquid. The infectivity of the tissue and of the culture liquid reached a peak at about 4 to 5 hours, the curve then flattened out as a plateau until about 12 hours, whereafter it decreased. These results have been interpreted to mean that between approximately 2 to 4 hours infected cells gradually came into virus production and released virus at the rate of 10^2 to 10^3 ID50 per cell per 15 minutes. Between 5 to 12 hours many cells underwent a cytopathogenic change as a result of infection and the decreased rate of virus production which resulted, balanced the thermal inactivation rate. After 12 hours most infected cells had ceased to be virus producers and the curve was predominantly that produced by thermal inactivation. A feature which may prove to be of considerable fundamental interest concerned the infectivity of cells during the latent period. It was found that if cells collected during their latent period were disrupted in phosphate buffer solution or culture medium, the suspension centrifuged and the resulting supernatant titrated, little or no infective virus could be detected. If, however, the cells debris following disruption was resuspended and the whole suspension titrated, a level of infectivity was obtained comparable to that of the number of cells that had been infected. Examination of the literature has shown that failure to detect infective virus in the supernatant of a centrifuged suspension of disrupted cells has sometimes been taken to indicate the occurence of an eclipse phase during the multiplication of a virus similar to that described with phages. Our results showed that a

considerable difference existed in the ease with which virus could be extracted or eluted from disrupted cells during the later stages of multiplication compared with during the latent period. The presence of such firmly bound infective virus in the cell debris may suggest, at least, that not all intracellular virus becomes non-infective during the latent period and possibily even that a phase of non-infectivity is not an essential requirement before viral synthesis can begin.

Monolayer cultures

Attempts to carry out differential growth curves using tongue epithelial cell suspensions, in which at frequent intervals the media was removed for titration, the cells thoroughly washed and resuspended in fresh media, were largely unsuccessful, as it was found that the cells would not survive the centrifugation and washing procedures. A search was therefore made for a suitable tissue to culture as a cell monolayer on glass with which experiments of this type could be made. This was done at first with monolayer culture of bovine amniotic ectodermal cells (Pay, 1956) and more recently with monolayer cultures of adult cattle tongue epithelial cells (Pay, 1957).

Results obtained with amnion cultures showed that following infection with seed virus of high titre, theoretically capable of infecting all susceptible cells with a multiplicity of more than 10 ID50, virus was first released into the culture medium at about 3 hours after infection and continued to be released at a steady rate in some experiments for at least 60 hours. Similar experiments using monolayer cultures of tongue epithelial cells showed that virus was released at a steady rate for the first 48 hours or so after infection and then at a gradually decreasing rate, so that by 96 hours the culture fluid had usually become non-infective. The quantitative study of virus multiplication by the differential growth curve in monolayer cultures of bovine tongue epithelium was complicated by the occurrence of two quite distinct types of cell growth which behaved very differently after infection. Cultures produced from trypsinised suspensions containing predominantly single, dispersed cells gave rise to a growth of discrete, polygonal shaped cells morphologically resembling those seen in cultures of kidney and Hela cells. These cells appeared to support the growth of virus without showing any apparent cytopathogenic effect. Furthermore, it was found that cultures of such cells, after producing virus and becoming non-infective again, could be repeatedly re-infected at intervals as short as 7 days and produce almost as much virus at each subsequent infection as they did initially. This result was also obtained when the cultures were maintained between subsequent infections, in a medium containing sufficient colchicine to inhibit mitosis and prevent the development of a new cell population.

Cultures produced from trypsinised suspensions containing clusters of cells as well as single cells gave rise to a totally distinct type of growth. Around the cell clumps then attached to the glass, a zone of cells developed which formed a more or less continuous "membrane" morphologically resembling typical pavement epithelium. This type of growth appeared to develop mainly by migration of cells from the clusters as, unlike the "polygonal" cell type of growth, it could be produced in a medium shown to inhibit mitosis. After infection with foot-and-mouth disease virus, the "membrane" cells showed most striking cytopathogenic changes. Within a few hours the cells began to retract from one another and the sheet became fenestrated. By 4 to 5 hours after infection many cells started to produce large, bulbous cytoplasmic protusions, often arranged radially around the cell and giving it an "aster" appearance. These protusions were never observed to burst or to detach from the cell, but appeared to coalesce gradually. Finally a large, spherical ballooned cell was produced, which resembled in some ways the cell showing hydropic degeneration found in the early stages of a developing vesicle in the epithelium of the infected animal. Similar changes have been described recently by Wesslén and Dinter (1956) in plasma clot cultures of bovine embryonic tongue and skin epithelium infected with foot-and-mouth disease virus.

Diagnosis and Typing

So far, tissue culture methods have not been used very widely for the diagnosis of foot-and-mouth disease as convenient alternative methods exist. The presence of foot-and-mouth disease virus in field samples is usually detected either by a direct complement fixation test or by isolation of the virus in unweaned mice followed by a complement fixation test. Occasionally, however, it has been found that material that fails to give a positive result by either of these two methods, will do so after passage in surviving tongue epithelial cultures (Brooksby, 1956).

Sellers (1956) found that it was possible to use pig kidney monolayer cultures for the identification and typing of foot-and-mouth disease virus. The presence of virus in a field sample was detected by the cytopathogenic effect produced when passaged in kidney cultures. The passaged virus was then typed by inoculation of duplicate kidney cultures after mixing with the various type immune sera; protection from cytopathogenic effects being given only by the homologous type serum.

References

BACHRACH, H. L., HESS, W. R., & CALLIS, J. J. (1955). *Science.* **122.** 1,269.

BROOKSBY, J. B. (1956). Personal communication.

———, & WARDLE, E. (1954). *J. Hyg. (Camb.).* **52.** 87.

CARTWRIGHT, S. F., PAY, T. W. F., & HENDERSON, W. M. (1957). *J. gen. Microbiol.* **16.** 730.

DULBECCO, R. (1952). *Proc. nat. Acad. Sci. Wash.* **38.** 747.

———. (1953). *J. exp. Med.* **99.** 167.

FRENKEL, H. S. (1947). *Bull. Off. int. Epiz.* **28.** 155.

———. (1953). *Ibid.* **39.** 91.

———. (1954). *Amer. J. vet. Res.* **15.** 346.

———, & RIBELIN, W. E. (1956). *Ibid.* **17.** 40.

GILLESPIE, J. H., & FRENKEL, H. S. (1955). *Ibid.* **16.** 505.

HECKE, F. (1930). *Zbl. Bakt. (Abt. I Orig.).* **116.** 386.

———. (1931). *Ibid.* **119.** 385.

HENDERSON, W. M. (1953a). In "The Nature of Virus Multiplication." *Sym. Soc. Gen. Microbiol.* **2.** 317.

———. (1953b). *Proc. 15th Int. vet. Congr., Stockholm, Part I.* p. 191.

———, & GALLOWAY, I. A. (1953). *J. Hyg. (Camb.).* **51.** 546.

(Concluded at foot of col. 1, overleaf)

The Use of Proteolytic Enzymes in Mastitis in Dairy Cattle

BY
W. J. JORDAN
Rossett

SUMMARY. — *Streptokinase-streptodornase, papain, crystalline trypsin, and stabilised crystalline trypsin were used in the treatment of mastitis in dairy cattle which had resisted a course of antibiotic treatment.*

Streptokinase-streptodornase improved 2 out of 6 summer mastitis cases treated. Papain has no noticeable effect. Crystalline trypsin was found a most useful therapeutic agent.

Introduction

THERE seems little doubt that many failures in the treatment of mastitis have been due to failure of penetration of the therapeutic agent rather than to insensitivity of the causal organism. This imperviousness is attributable in part to inflammatory reaction but also in many instances to the accumulation of semi-solid secretion in the ducts of the gland.

On this assumption it was decided to test the clinical effects of proteolytic enzymes when used in conjunction with appropriate antibiotics, in cases where treatment with antibiotics alone had produced no clinical improvement. The first series of cases was treated in 1954 using streptokinase-streptodornase. In the following year papain was tried. In 1956 a preparation of pure trypsin was kindly offered for trial by a Danish colleague. This year a stabilised form of pure trypsin has been made available. Almost all dairy breeds of cattle were included in this trial.

Method and Materials

Streptokinase-streptodornase

Supplies of this were limited so only 6 cases of summer mastitis in dry cows were treated. In all cases one quarter only was affected. The affected quarter was stripped out and a sample of secretion taken for microscopic examination. The contents of one bottle (20,000 units Christensen S.K. 5,000 units S.D.) dissolved in 10 c.c. sterile saline solution were then infused into the quarter *via* the teat canal, followed by one tube of 300,000 I.U. procaine penicillin, and the gland massaged. The quarter was stripped out 24 hours later and again at 48 hours, and on both occasions further infusions of streptokinase-streptodornase and procaine penicillin were made.

Papain

As this is only partially soluble in water and liable to deterioration through oxidation, it was made up in mineral oil (10 grains in each intramammary tube for ease of administration). It had the disadvantage of curdling milk. Six cases of chronic mastitis were treated by stripping out the affected quarter and injecting, *via* the teat canal, the contents of one tube of papain, one tube of a mixture of 100,000 I.U. of penicillin, and 250,000 I.U. streptomycin. This was repeated the following day.

Trypsin

This was supplied in vials containing 50 mg. of powdered crystalline trypsin standardised to 25 Anson trypsin units per gramme, together with 20-ml. vials containing 15 ml. of sterile isotonic diluent. The 63 cases of mastitis treated with trypsin had one common factor, namely, little or no secretion could be drawn from the gland. They fell roughly into 3 main groups:—

Acute mastitis, where the condition was of sudden onset and the gland was tense and painful.

Chronic mastitis, where the disease had been active for some time, and there was induration of the gland with diminished, and often thick, secretion.

Summer mastitis of dry cows.

Crystalline trypsin was not stable, and once prepared by dissolving in the diluent would only keep a few hours in the vial, but longer if brought into contact with necrotic tissue. This instability was due to autodigestion which became infinitesimal in the presence of necrotic tissue. It was prepared, therefore, immediately before use. It was given in 3 ways, *viz.* intravenously, intramuscularly, and intramammary. Two cows each had the contents of one vial intravenously and 5 cows each one vial intramuscularly. One of the 2, and 3 of the 5, received in addition half the contents of one vial by intramammary infusion. That is to say, of all the cows treated

Symposium of Viruses.—*Concluded.*

MACKOWIAK, C., GIRARD, H., CAMAND, R., & HIRTZ, J. (1955). *Rev. Immunol.* **19.** 426.
MAITLAND, M. C., & MAITLAND, H. B. (1931). *J. comp. Path.* **44.** 106.
MURRAY, M. R., & KOPECH, G. (1953). "A Bibliography of the Research in Tissue Culture." Vol. II. Academic Press Inc., New York. 1,703.
NARDELLI, L., & PERINI, E. (1956). *Vet. ital.* **7.** 321.
———, & ———. (1956). *Vet. Bull.* **26.** 2,588.
PAY, T. W. F. (1956). *Nature, Lond.* **177.** 752.
———. (1957). *Proc. R. Soc. Med.* **50.** 919.
PETERMAN, H. G., LANG, R., & MACKOWIAK, C. (1956). *C.R. Acad. Sci., Paris.* **243.** 991.
SELLERS, R. F. (1955). *Nature, Lond.* **176.** 547.
———. (1956). Personal communication.
———. (1957). *Proc. R. Soc. Med.* **50.** 915.
SKINNER, H. H. (1951). *Ibid.* **44.** 1,041.
UBERTINI, B., NARDELLI, L., BAREI, S., & SANTERO, G. (1956). *Zbl. VetMed.* **3.** 419.
VAN BEKKUM, J. G. (1955). Report. Amsterdam.
WESSLEN, T., & DINTER, Z. (1956). *Nord. VetMed.* **10.** 795.
WILLEMS, R., & LEUNEN, J. (1956). *Bull. Off. int. Epiz.* **45.** 298.

(To be continued)

with trypsin, only 3 did not receive it by intramammary infusion. The remaining 60 cows were infused with trypsin solution in varying doses and frequency. In the first trials one vial was given once per day, then twice daily, and finally the majority of cases received half a vial twice daily. Three of the summer mastitis cases and two cases of chronic mastitis received half a vial 3 times daily for 2 days. All these animals continued to receive appropriate antibiotic therapy by intramammary infusion as indicated by microscopic examination of the secretion. The summer mastitis cases received daily 2 grammes streptomycin and 3,000,000 I.U. penicillin intramuscularly plus 2 grammes streptomycin (as sulphate) and 1,000,000 I.U. procaine penicillin in an oily gel intramammary.

Stabilised crystalline trypsin, 50 mg. per vial containing 25 Anson units per gramme, was used in 7 cases of chronic mastitis this year. Each case received, intramammary, the contents of one vial each day for 3 days. The quarter was stripped out first and then again 8 hours later, at which time the antibiotic was injected into the gland *via* the teat canal.

Results

Streptokinase-streptodornase

Of 6 cases of summer mastitis in dry cows only 2 showed improvement, but in neither case did the gland secrete much after calving. There was no toxic reaction.

Papain

Of the 6 cases of chronic mastitis treated none showed any improvement that could be attributed to the papain. There was no apparent toxic reaction.

Crystalline trypsin

Of the 7 cases of summer mastitis treated 3 showed definite improvement, and 2 of these cases have since given a normal quantity of milk on what were the diseased quarters. Of 56 cases of mastitis in milking cows, 40 showed marked improvement. This was particularly noticeable in chronic mastitis where, after only one dose, the secretion, which had been sparse, thick, or even inspissated, became more fluid and copious. The quarter could be stripped more easily. There was no doubt that the proteolytic enzyme was at work because softening began at the base of the teat and spread into the gland, the last part of the gland to soften being farthest from the teat.

Some of the remaining 16 cases may have shown some slight improvement but the mastitis could not be cleared. Most of the 40 cows which responded well were cleared of mastitis, and those have since calved again and are milking normally. In the 2 cases treated by intravenous injection no effect, either curative or toxic, was observed. In the 5 cows treated by intramuscular injection it was thought in 3 cases that softening had occurred high up in the gland. No toxic effect was noted.

Stabilised crystalline trypsin

Of 7 cases so far treated, 4 have shown improvement.

Enzyme	Mastitis in milking cows		Summer mastitis in dry cows	
	Number treated	Number improved	Number treated	Number improved
Streptokinase-streptodornase	—	—	6	2
Papain	6	—	—	—
Trypsin	56	40	7	3
Stabilised Trypsin	7	4	—	—

Discussion

There is little else to be said about the use of streptokinase and papain.

In this series we had to ascertain first if the enzyme on trial was, in fact, effective and, if so, in what quantity. Also, how frequent the dosing should be and the best mode of administration. Although we had the impression that there may have been some proteolytic activity in the affected quarter following intramuscular injection, this was not clear-cut and intrammary infusion has proved the method of choice. By trial and error it was found that 25 mg. crystalline trypsin twice daily, stripping the quarter immediately before each infusion, gave the most consistent results. Fifty mg. infused once daily, stripping out 8 to 12 hours later, was the best method with the stabilised form of trypsin. Even after one infusion, at the next stripping the amount of secretion had markedly increased, and softening had begun around the base of the teat. Later this softening spread fanwise into the gland and the secretion progressively increased.

There were failures. Some of these may have been due to early inexpert handling of the enzyme. Some were probably due to the fact that in long-established cases of chronic mastitis much of the indurated tissue is fibriotic, *i.e.* living tissue which is protected by trypsin inhibitors.

One of the initial disadvantages was the instability of the enzyme, which necessitated visiting cases twice or three times daily. This has now been overcome by the production of a stabilised form.

Acknowledgements.—My thanks are due to Burroughs Wellcome for the supplies of streptokinase-streptodornase, to Consolidated Chemicals for the supplies of papain, to Novo Terapeutisk Laboratorium, Copenhagen, for the supplies of crystalline trypsin, and to Evans Medical Supplies Limited for the supplies of stabilised crystalline trypsin.

Addendum

Since this paper was written a further series of cases have been treated, some with paratrypsin and some with Deanase, with favourable results. Both these were kindly supplied by Consolidated Chemicals.

Current Literature

ABSTRACTS

Displaced Abomasum a Relatively Common Bovine Syndrome. HANSEN *et al.* (1957). *Amer. Vet.* **38.** 129-34.

The majority of the 40 cases of abomasal displacement seen by these authors have occurred either in the late stage of pregnancy or within 2 weeks of calving. Anorexia and a drop in milk yield are the initial clinical findings. The most significant symptoms are (1) the distension of the left sublumbar fossa by the gas-filled abomasum, (2) the presence of high-pitched sounds detected by auscultation of the upper flank close to the last rib, and (3) the complete atony of the rumen.

Surgical treatment has comprised laparotomy *via* the left flank, under paravertebral anaesthesia. Once the abomasum is deflated, by puncture with a 12-gauge hypodermic needle, it is possible for it to be pushed down below the rumen and so revert to its normal position.

Within from 48 hours to several weeks the displacement has recurred in many instances. Recurrence, however, has been successfully prevented in 5 recent cases by an additional surgical manoeuvre. After the abomasal displacement has been corrected a rumenotomy is performed and a suture of non-absorbable material is so placed through the floor of the rumen and abdomen that 4 to 6 inches of these structures are involved in a longitudinal direction. The suture is tied on the outside and removed after 7 days.

L. C. V.

Combined Thyroid-Androgen-Estrogen Therapy in Canine Practice. GREENE, J. G. (1957). *Vet. Med.* **52.** 395-6.

The effects of a "metabolic regulator" on 50 canine patients has been assessed. The special treatment was the use of an anabolin* made up in tablets containing 2.5 mg. methyltestosterone, 0.005 mg. ethinyl estradiol, and ¼ g. proloid (Thyroglobulin, Warner-Chilcott Laboratories). It is suggested that the various components of the anabolin act synergistically, possibly at the cellular level, to restore the physiological processes of normal equilibrium, for example the nitrogen and calcium levels.

The author describes the results of this therapy in (a) 15 young dogs recovering from virus infections and (b) in 35 older somewhat senile dogs. The adult dogs, 8 to 12 years old, showed symptoms of old age including indigestion, posterior weakness, alopecia, poor teeth, loss of condition, and general malaise. The young dogs were less than 2 years but were convalescing from virus infections and showed symptoms of anorexia, poor coat, and thinness.

Average sized dogs received 1 tablet and large dogs 2 tablets daily. Improvement was shown in 7 to 10 days and continued steadily over several weeks. 95 per cent. of the treated cases showed satisfactory results manifest as increase in muscle tone and vigour, loss of urinary incontinence, and correction of faulty hair growth. A few cases which relapsed showed immediate recovery when the therapy was recommenced. The treatment was well tolerated.

F. R. B.

* Warner-Chilcott Laboratories, Morris Plains, N.J., U.S.A.

News and Comment

A. H. T. NINTH REPORT

Inflation and the increased cost of living have had some effect on the educational activities of the Animal Health Trust, particularly in the case of undergraduate bursaries for which there have been an increasing number of applications, some of which have had to be refused owing to financial limitations. Similarly, it has been found necessary to curtail the actual number of post-graduate scholarships and research fellowships, mainly owing to the fact that individual grants have had to be raised in order to meet additional costs.

These facts are disclosed in the Trust's recently published Ninth Report in which it is stated that, in the 15 years of the Trust's existence, a total of well over 300 educational awards of all kinds have been made at an over-all cost of nearly £105,000.

Commenting on the inflationary situation as it affects undergraduate bursaries, the Report says: "The undergraduate work of the Trust is undoubtedly filling a real need, particularly to those parents—largely professional men and women—whose income is not sufficient to pay the present level of fees for their children at universities, and yet is such that it precludes the children from getting normal State aid."

Of the total of 88 post-graduate awards made so far by the Trust, 76 have gone to young veterinarians and the remainder to graduates in allied sciences. It is interesting to note that the majority of scholars chose pathology, parasitology, or bacteriology, while relatively few wished to specialise in animal husbandry, genetics, and nutrition. The actual numbers of scholars following the various disciplines were: pathology, 17; parasitology, 15; physiology (including infertility), 11; bacteriology, 9; biochemistry, 18; nutrition, 2; medicine and surgery, 3 each; animal husbandry, 2; genetics, animals kept in zoos, and game birds, 1 each.

The Report, which contains reviews of the scientific work carried out during the past 2 years by all 4 of the Trust's research stations, also provides up-to-date information on administration, finance, and personnel. Copies (sixpence each) are available from: The General Secretary, The Animal Health Trust, 14, Ashley Place, London, S.W.1.

MIDDLETON MEMORIAL LECTURE

The 5th Middleton Memorial Lecture was given in London on December 5th by Dr. Alan Robertson, of the Institute of Genetics, Edinburgh University.

Dr. Robertson said he would be happy to see the end of bull and boar licensing, not because it was actively harmful, but because it meant that the efforts of livestock officers were directed to something useless, for as a result some 10 per cent. of bulls, coming mainly from the bottom of the breed structure, were refused licences on grounds that had nothing to do with their merit as commercial stock-getters. Dr. Robertson stressed the point that A.I. presented a magnificent opportunity for progeny testing, but said that some writers' suggestion that by continued in-breeding and selection it should be possible to produce a superior homozygous line was apparently a myth, as nobody had yet succeeded in producing such a line.

The Middleton Memorial lectures are a memorial to Sir Thomas Middleton, chief of the Ministry of Agriculture in London during the First World War. The Chairman at Dr. Robertson's lecture was Professor R. G. White, ex-director of the Agricultural Research Council's Animal Breeding Research Organisation.

R.C.V.S. EXAMINATIONS

EDINBURGH—FINAL EXAMINATION

Clark, James; Hartley, E. G; Rodger, J. L.; and Robertson, H. L.

DUBLIN—THIRD EXAMINATION

Pathology only

Barrett, J. N.; Berry, J. P.; Corry, D. M. G.; Delahunty, D.; Dowling, W. A.; Hannigan, J. H.; Hernon, John; Holloway, A. B.; Lawlor, Florence; Lowndes, J. D.; Lydon, C. T. J.; McDonald, W. E.; McKervey, J. H.; Moloney, Richard; O'Brien, T. J.; O'Leary, Michael; O'Reilly, D. J.; Reidy, D. F.; Sweetman, W. L.; Trouton, R. K.; Walsh, John; Wilson, R. W.

FINAL EXAMINATION

Carroll, Denis; Costello, E. A.; Denvir, K.; Downey, J. S.; Flanagan, J. P.; Hoban, G. P.; Johnston, J. B. H.; Kelleher, J. F.; King, M. B.; McGovern, P. G.; May, D. J.; Monaghan, M. G. B.; Osborne, M.

LONDON—THIRD EXAMINATION

Pathology only

Ali, Sadiq

FINAL EXAMINATION

Cullinane, J. A.; Howse, J. N.; Tanner, J. A.

LIVERPOOL—THIRD EXAMINATION

Pathology only

Yusuf, Muhammed.

COMING EVENTS

January

2nd (Thurs.). General Meeting of the Central Veterinary Society at the Royal Veterinary College, Camden Town, N.W.1, 5.30 p.m.

8th, 9th and 10th (Wed., Thurs. and Fri.). R.C.V.S. Committee and Council Meetings.

17th (Fri.). Annual Buffet-Supper Dance of the Scottish Metropolitan Division, V.V.B.F. Ladies' Guild, 8 p.m.

25th (Fri.). Annual General Meeting of the Mid-west Division, Berkeley Café, Bristol, 2.15 p.m.

February

7th (Fri.). Annual Ball of the Royal (Dick) School of Veterinary Studies, in the Assembly Rooms, George Street, Edinburgh, 9 p.m.

B.V.A. COUNCIL AND COMMITTEE MEETINGS

Wednesday, January 22nd, 1958, at No. 7, Mansfield Street, W.1.

10.00 a.m. Veterinary State Medicine Committee.
11.30 a.m. Small-Animals Committee.
2.00 p.m. Farm Livestock Committee.

Thursday, January 23rd, 1958, at No. 7, Mansfield Street, W.1.

10.00 a.m. Parliamentary and Public Relations Committee.
11.30 a.m. Home Appointments Committee.
12.30 p.m. Finance Sub-committee.
2.15 p.m. General Purposes and Finance Committee.

Friday, January 24th, 1958, at the Connaught Rooms, W.C.2.

10.30 a.m. Council Meeting.

PERSONAL

Birth

HESKETH.—On November 27th, 1957, to Elizabeth, wife of William C. Hesketh, M.R.C.V.S., of Torquay, a son, Robert John.

Engagement

STOKES—THOMPSON.—The engagement is announced between Peter Martin Stokes, M.R.C.V.S., only son of the late Mr. and Mrs. W. N. Stokes of Melbourn, Cambs., and Jean Mary Thompson, only daughter of Mr. and Mrs. H. A. C. Thompson of Worthing, Sussex.

CORRESPONDENCE

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A.

Foot-rot in Sheep

Sir,—Mr. Jones Pugh's letter has the merit of drawing the attention of practitioners to the vast Australian experience of this condition; and the demerit of quoting out of context. It is admittedly a counsel of perfection to suggest that those interested in this disease should read for themselves the papers recently published in the *Australian Veterinary Journal* since this is not always readily available. But in the absence of relevant abstracts in THE VETERINARY RECORD it is difficult to see how else practitioners are to assess this work.

One of the most significant papers on this matter is that by Forsyth (*Aust. vet. J.*, July, 1957), on the use of a 20 per cent. solution of Cetrimide in alcohol, of which an abstract was sent to you. It was thought of sufficient interest to merit a review, but was not published.

In opening the discussion to the paper quoted by

Mr. Jones Pugh, Mr. Toop said: " 20 per cent. cetyl trimethyl ammonium bromide (Cetrimide) in alcohol has only recently become available in Western Australia and the very limited observations it has been possible to make suggest that it is about equal in efficiency to chloromycetin and at about one-tenth of the cost." Forsyth's conclusions are similar. Different climates alter cases. It would be foolish to expect a replication of Australian experience, in all aspects of this condition, under British conditions. It is clear, however, that in two respects at least we should benefit from this experience, including as it does the worst outbreak of foot-rot (1956) in Australian history. Firstly, the preliminary surgical toilette of the affected feet must be " heroic " in thoroughness. Secondly, successful control requires the most efficient foot dressing (in terms of cost/cure) to be available to sheep farmers economically enough to ensure its adequate use.

Yours faithfully,
G. V. SHORT,
Veterinary Department.

Imperial Chemical Industries Limited,
Fulshaw Hall,
Wilmslow,
Cheshire.

December 17th, 1957.

The " Rights " of Animals

Sir,—All people with a humane spark in their constitution must feel distress and disgust at the fiendish action of their fellowmen in subjecting an innocent and dumb animal to the torture the " space dog " has undoubtedly endured.

Mr. A. L. Bongard has stated in his letter to THE RECORD that the Prince of Denmark in his superlative eulogy of man describes him thus: " What a piece of work is man! How noble in reason! How infinite in faculty! In form and moving how express and admirable! In action how like an angel! In apprehension how like a God! The beauty of the world! The paragon of animals!" But he forgot to say " and in whom Satan took up his permanent abode."

Many men and women of all nations and creeds deny any rights to animals, thereby considering themselves perfectly justified in subjecting them to all forms of cruelty if by so doing their sadistic desires are attained. Many are ignorant of and, if not, ignore the fact that the composition of all animals, including man, is an integration of organic and inorganic matter, assembled to a definite design in each animal species by that great Architect of Creation we call God. But God alone has the power to instil the spark that gives vitalisation to this assembly. Man has the power to destroy but is absolutely incapable of creating life. From whence it comes and whence it goes he is in abysmal ignorance. Yet in his supreme presumption and arrogance he endeavours to allocate to himself an infinite life after death and a complete denial of the same to the lower animals, thereby sitting in judgment on the design for the fulfilment of the Creator's ultimate purpose.

With the exception of the imbecile and insane all humans are endowed with a power to reason. If we define reason as " the faculty of the mind by which man draws conclusions and determines right and truth " it follows therefore that reason is the one attribute possessed by man which gives him supremacy over other animals; but at the same time it imposes upon him a duty in his attitude towards his fellow beings and all animals. By exercising reason we are capable of arriving at truth. If we define truth as " a spiritual thing, the image of God reflected in man," and if within our hearts we are possessed of truth, we have a directive which imposes upon us the obligation to recognise the insane, animals, and children, as God's especial care which as such should also be ours.

Men and women who scoff at those who endeavour to render care and rights to animals, arguing I presume, that any form of cruelty is justified if it enables them to attain their objective should remember the immortal words of the poet Gray:—

" The boast of heraldry, the pomp of pow'r,
And all that beauty, and all that wealth e'er gave
Await alike th' inevitable hour,
The paths of glory lead but to the grave."

Then they will be required to give an account of their stewardship while here on earth.

Yours faithfully,
L. C. MAGUIRE.

Boltown Hall,
Kilskyre,
Kells,
County Meath.

November 18th, 1957.

Circulars Through the Post

Sir,—I fully support Mr. Fuller's contention that the large amount of printed matter (often most expensively produced on art paper) dropped into our letter-boxes is not only a waste of paper and money, but becomes a great nuisance. Day after day I receive reams of paper, often advertising the same drugs over and over again, and which I regularly used when in active practice.

Most of the advertising matter pertains exclusively to large-animal practice, and as I am (or was) essentially a small-animal practitioner, such notices go straight into the waste-paper basket. Like other colleagues, I feel that most of the money expended in this wasteful way (with increased postal charges) would be better utilised in reducing the cost of the articles advertised, and so acceding to the Government's call to cut prices.

Surely all the information sent us through the post could reach us just as effectively through the advertisement pages of THE VETERINARY RECORD, and without cluttering up our letter-boxes?

Yours faithfully,
H. KIRK.

28, Park Hill,
Ealing, W.5.

December 16th, 1957.